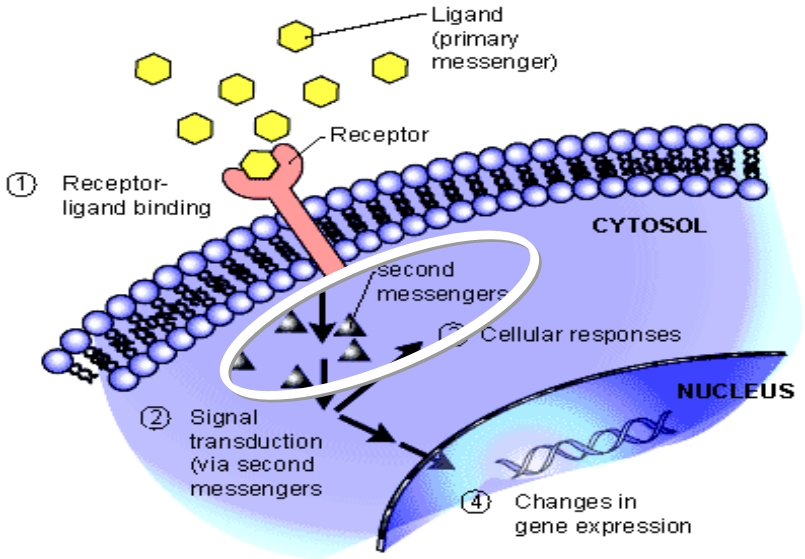
Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 17

Regulation of gene expression by second messengers other than cAMP



cAMP

cGMP

IP3

DAG

Ca^{2+}

Lectures 15 and 16

Introduction to signal transduction

Lipophilic signalling molecules and intracellular receptors

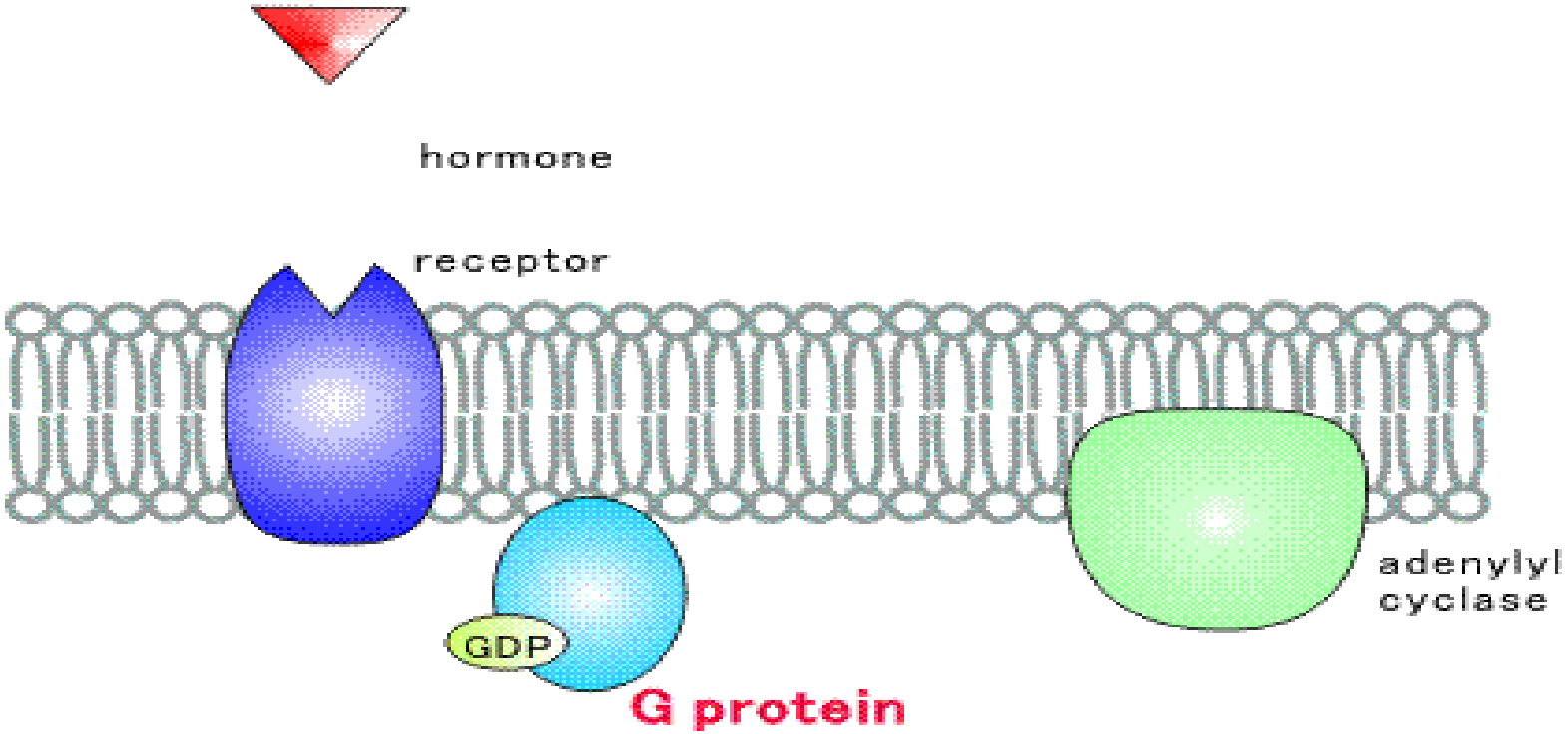
Water soluble signalling molecules and membrane receptors

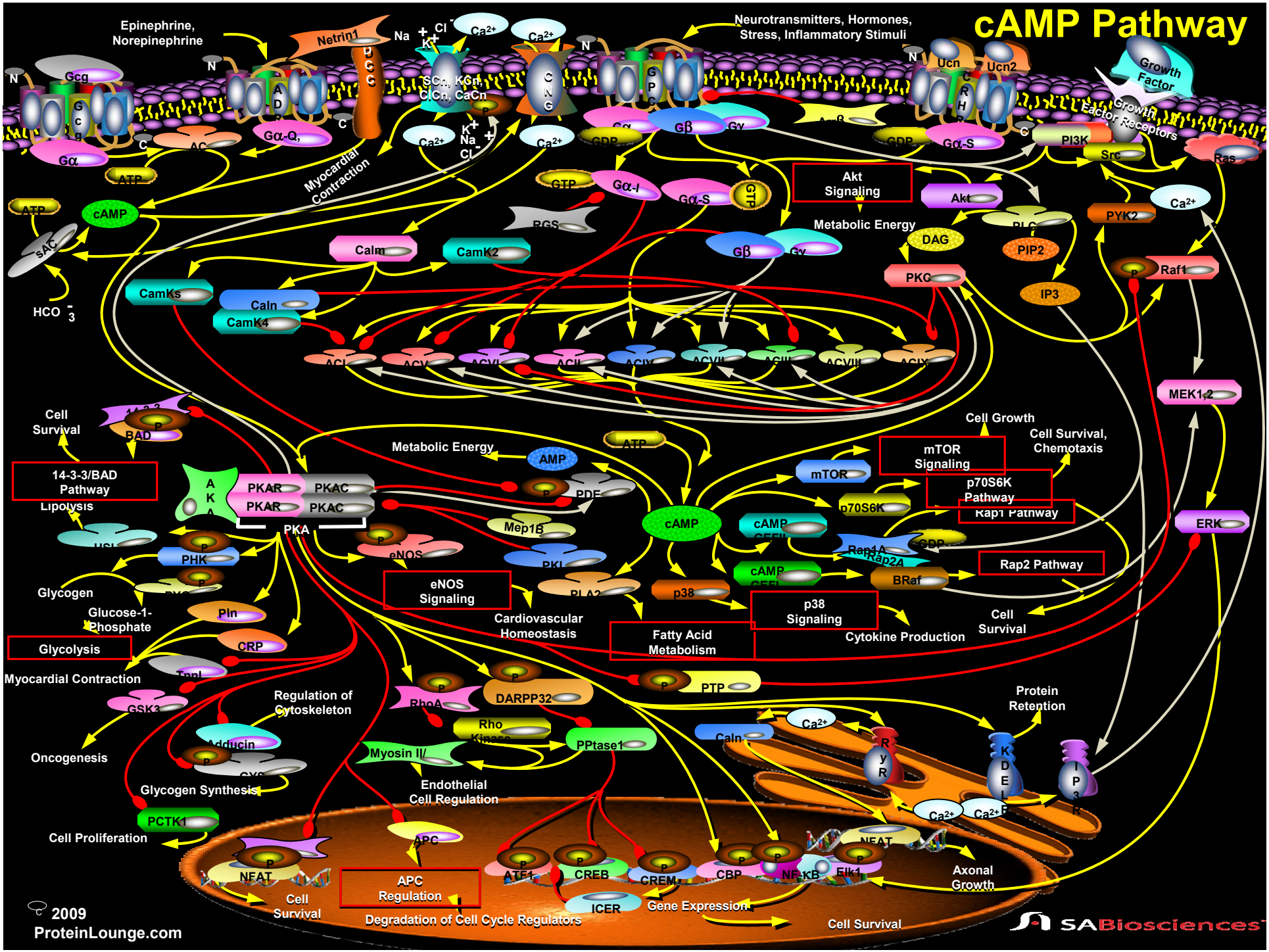
Activation of adenylate cyclase and synthesis of cAMP by GPCR signalling pathway involving trimeric G proteins

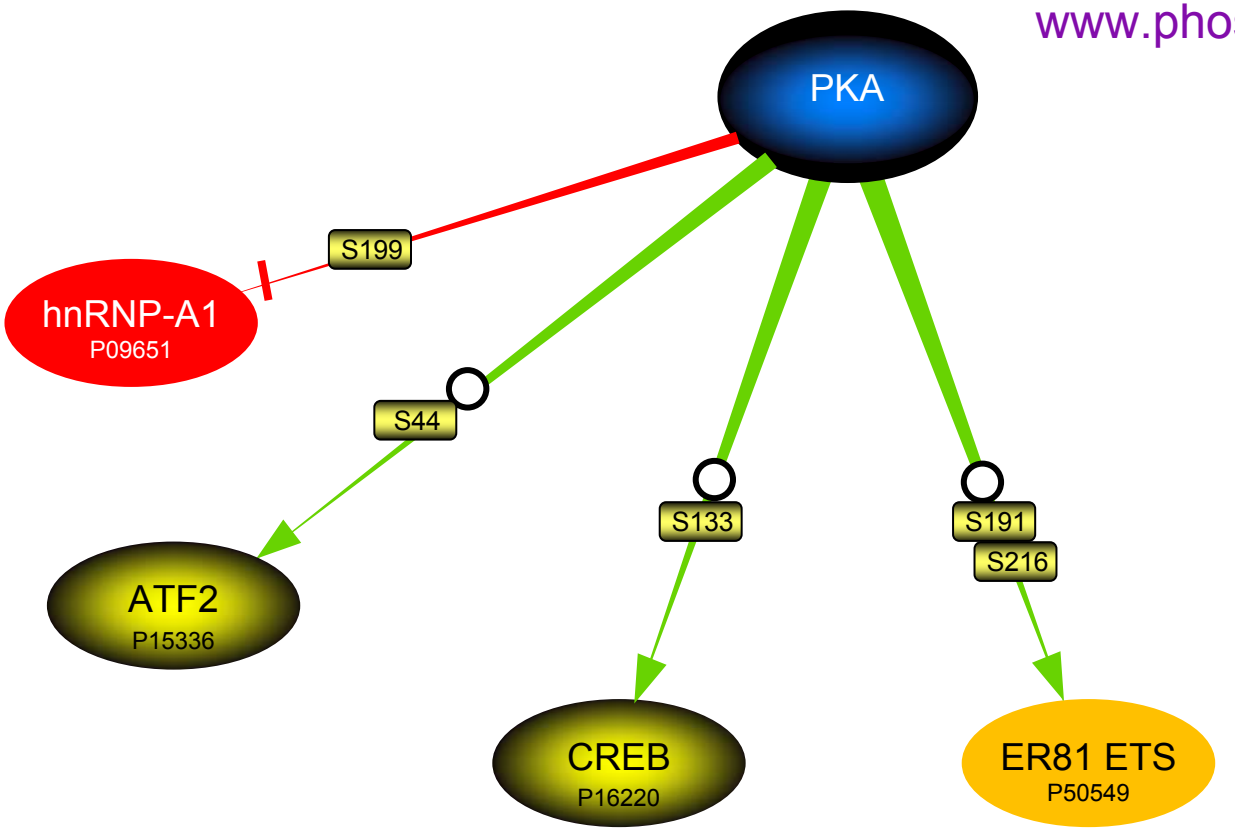
Synthesis of cAMP and activation of protein kinase A

Regulation of gene expression by cAMP and protein kinase A via ATF/CREB family of transcription factors

Regulation of gene expression by cGMP, IP3, DAG, Ca^{2+}


hormone
receptor
adenylyl
cyclase
GDP
G protein


cAMP Pathway
Epinephrine, Norepinephrine
Neurotransmitters, Hormones, Stress, Inflammatory Stimuli
Netrin1
Growth Factor
Growth Factor Receptors
Gcg
Ucn
Ucn2
Gα
Gβ
Gγ
Gα-Q,
Gα-S
Gα-I
GTP
GDP
ATP
cAMP
sAC
HCO 3
AC
PI3K
Src
Ras
Akt
Akt Signaling
Metabolic Energy
PYK2
Ca2+
PLC
DAG
PIP2
IP3
PKC
Raf1
MEK1,2
ERK
Calm
CamK2
CamKs
Caln
CamK4
RGS
Myocardial Contraction
Cell Survival
14-3-3/BAD Pathway
BAD
Lipolysis
PKA
PKAR
PKAC
AMP
PDE
PHK
Glycogen
Glucose-1-Phosphate
Glycolysis
Pln
CRP
TnnI
GSK3
Oncogenesis
Regulation of Cytoskeleton
Glycogen Synthesis
PCTK1
Cell Proliferation
Mep1β
eNOS
PKI
PLA2
eNOS Signaling
Cardiovascular Homeostasis
p38
p38 Signaling
Cytokine Production
Cell Survival
Fatty Acid Metabolism
mTOR
mTOR Signaling
Cell Growth
Cell Survival, Chemotaxis
p70S6K
p70S6K Pathway
Rap1 Pathway
Rap1A
Rap2A
Rap2 Pathway
BRaf
cAMP GEFI
cAMP GEFII
PTP
RhoA
DARPP32
Rho Kinase
PPtase1
Myosin II
Endothelial Cell Regulation
Adducin
Protein Retention
RyR
IP3R
KDEL R
Caln
Ca2+
NFAT
APC
APC Regulation
Degradation of Cell Cycle Regulators
ATF1
CREB
CREM
CBP
NF-κB
Elk1
ICER
Gene Expression
Axonal Growth
Cell Survival
2009
ProteinLounge.com
SABiosciences

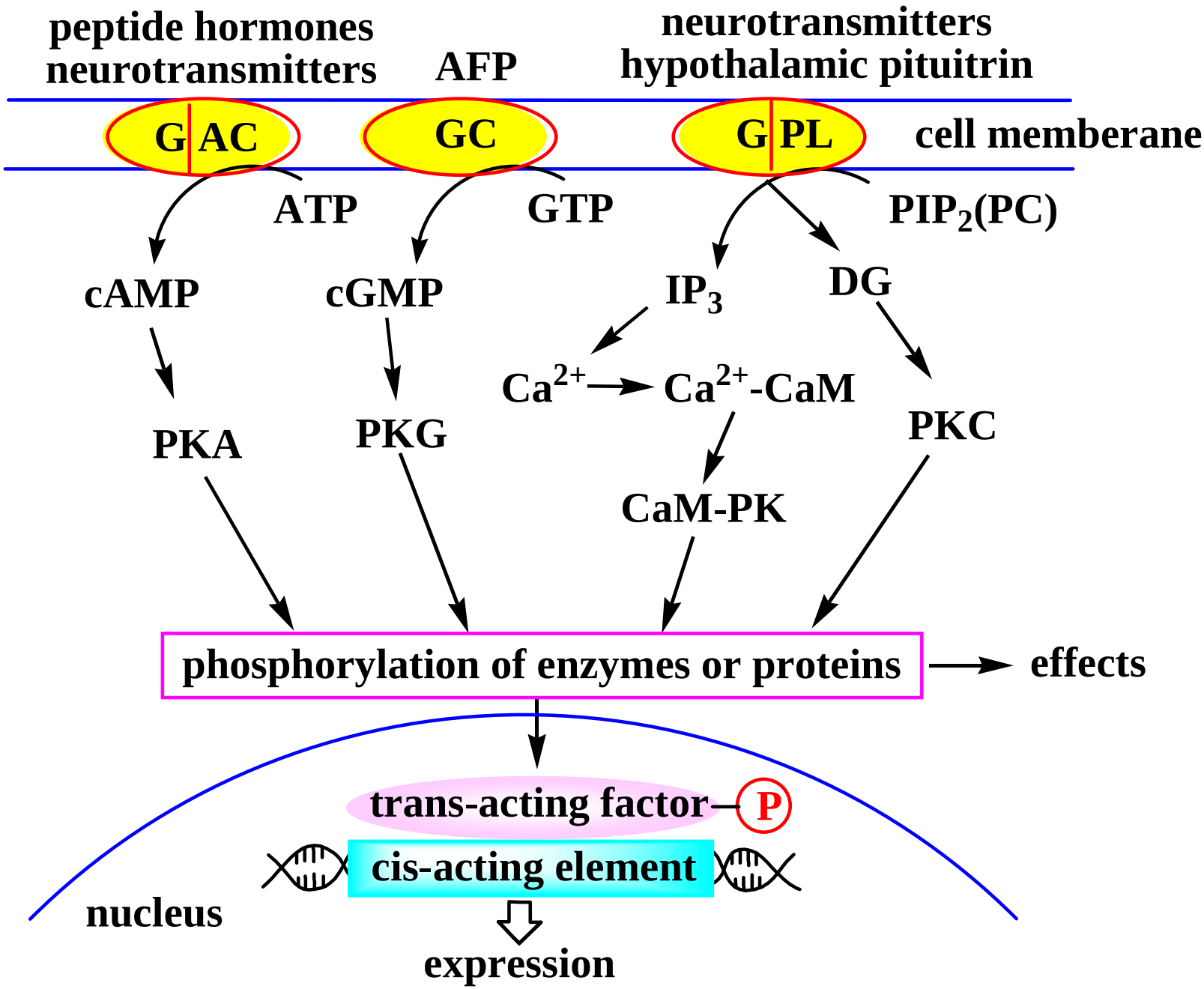


cAMP dependent-protein kinase A pathway

Today's lecture

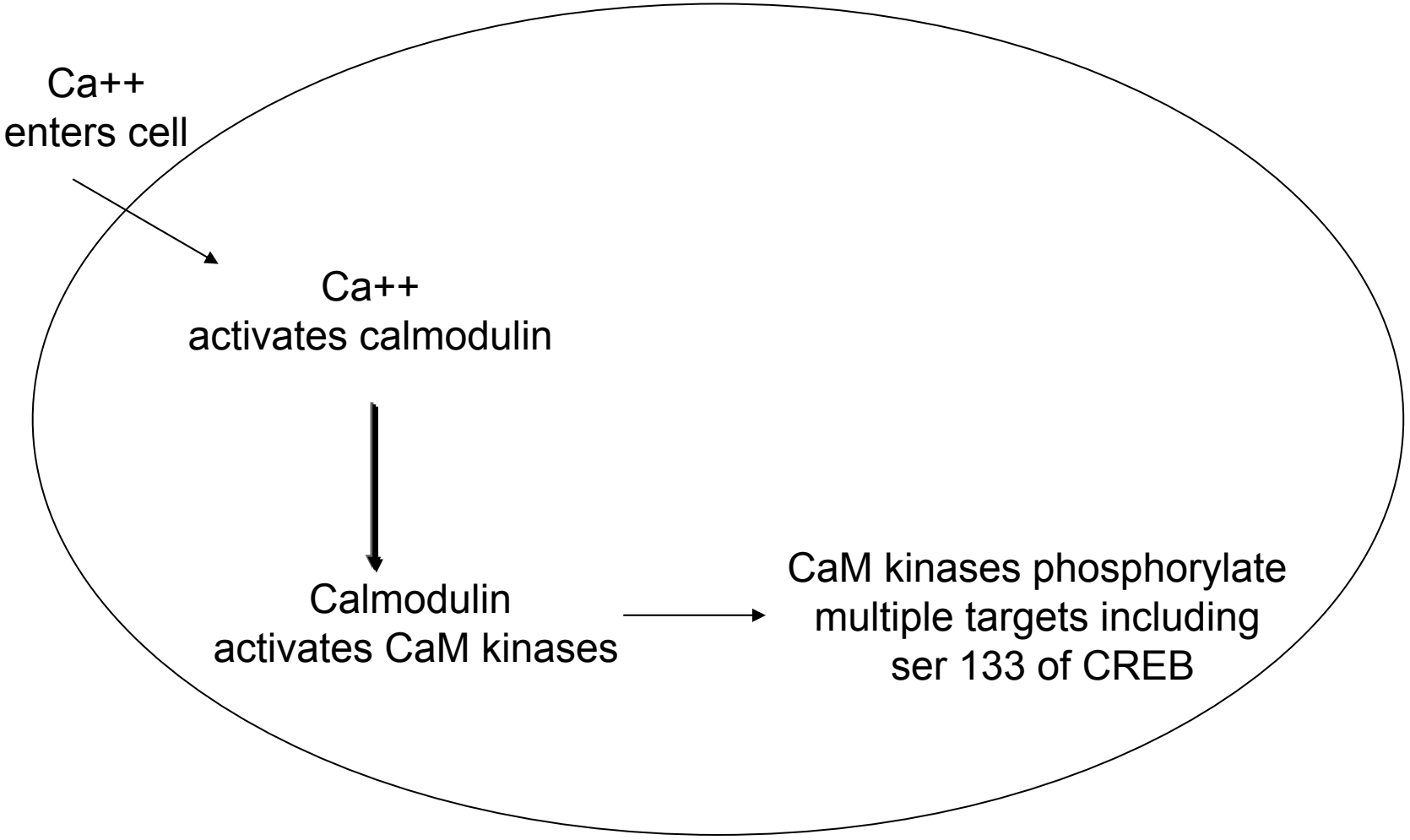
cGMP-dependent protein kinase G pathway

Ca^{2+}-CaMK and DAG-protein kinase C pathways


peptide hormones
neurotransmitters
AFP
neurotransmitters
hypothalamic pituitrin
G AC
GC
G PL
cell memberane
ATP
GTP
PIP2(PC)
cAMP
cGMP
IP3
DG
Ca2+
Ca2+-CaM
PKA
PKG
PKC
CaM-PK
phosphorylation of enzymes or proteins
effects
trans-acting factor
P
cis-acting element
nucleus
expression

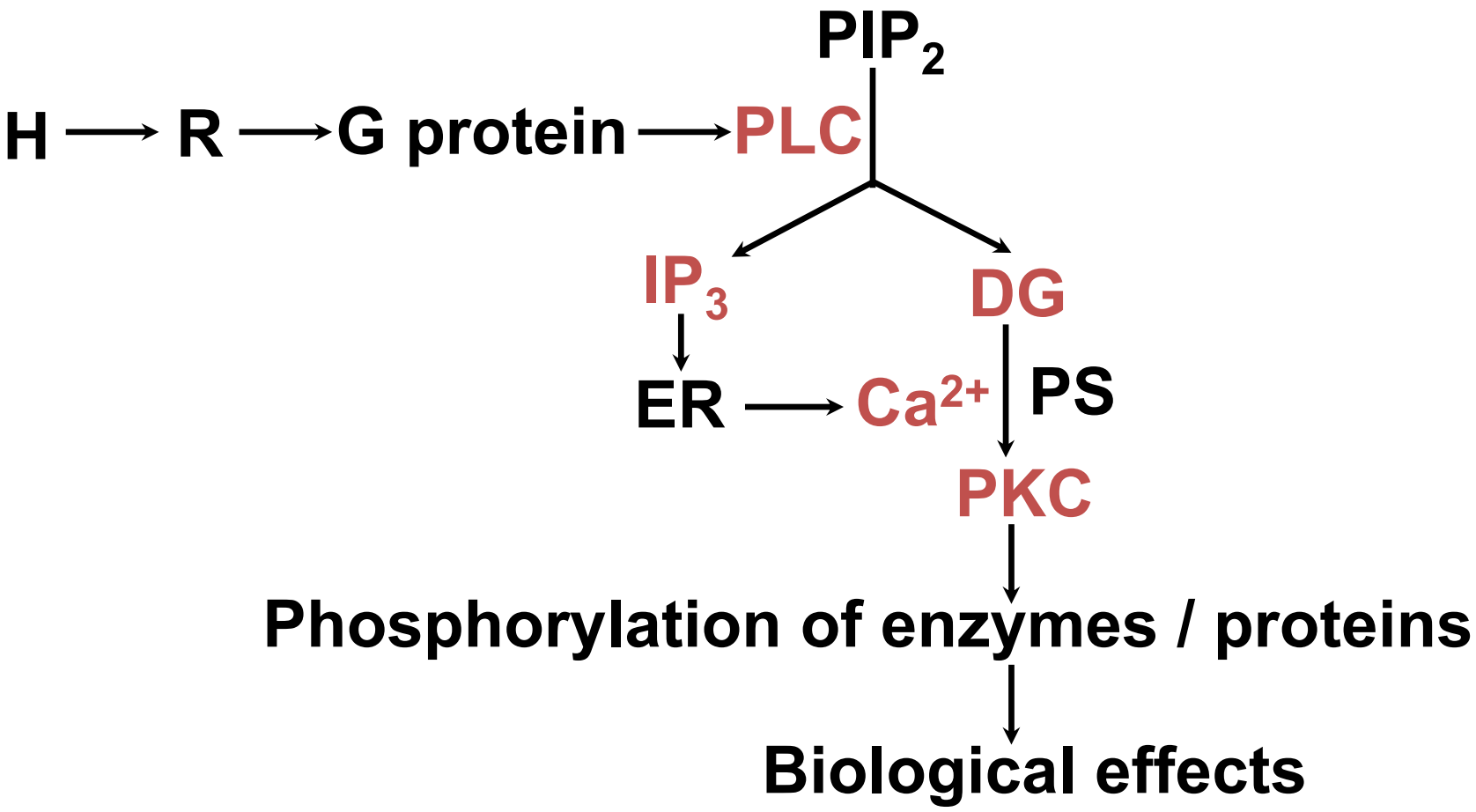
Ca^{2+} signalling

Calcium ions activate Calcium-Calmodulin Kinases (CaM Kinases) which phosphorylate CREB and thus activate genes containing CREs

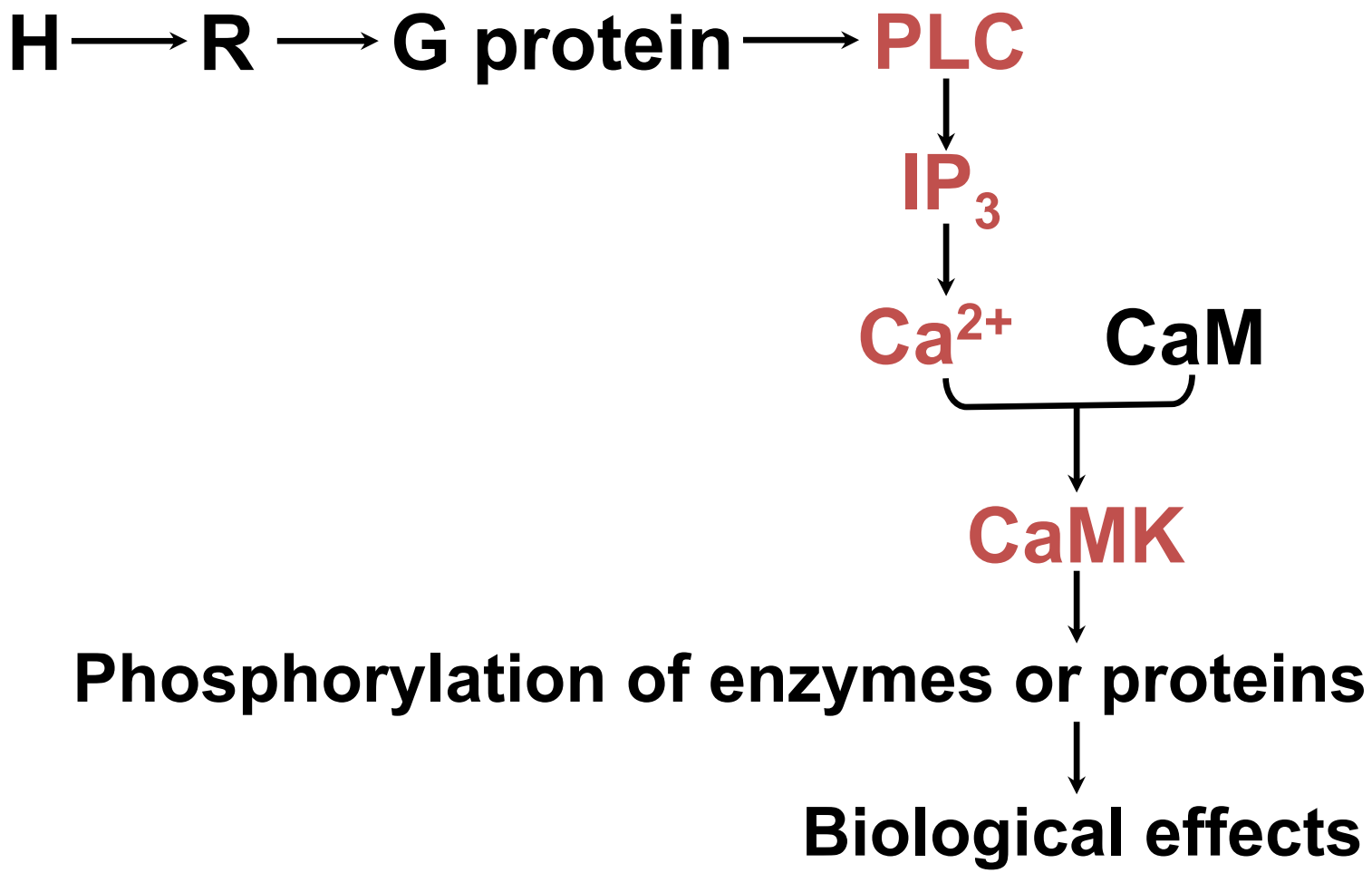


Ca^{2+} dependent protein kinase pathway

(1) Ca^{2+} -DAG -dependent PKC pathway



(2) Ca^{2+}-CaM dependent protein kinase pathway



Calmodulin (CaMKII)

Ca^{2+} binding protein

4 Ca^{2+} CaM → Ca^{2+}- CaM

↓

CaM kinase↑

↓

Ser/Thr - P

↓

Ca^{2+} pump, AC ↑ GC ↑

Enzymes (glycogen synthase, phosphorylase kinase)

Signalling Molecule

Receptor

Gs

Adenylate Cyclase

cAMP

PKA

cAMP PATHWAY

Signalling Molecule

Receptor

Gq

Phospholipase C

DAG and IP3

Ca^{2+} release, Calmodulin Kinases & Protein Kinase C

Ca^{2+} PATHWAY

cGMP signalling

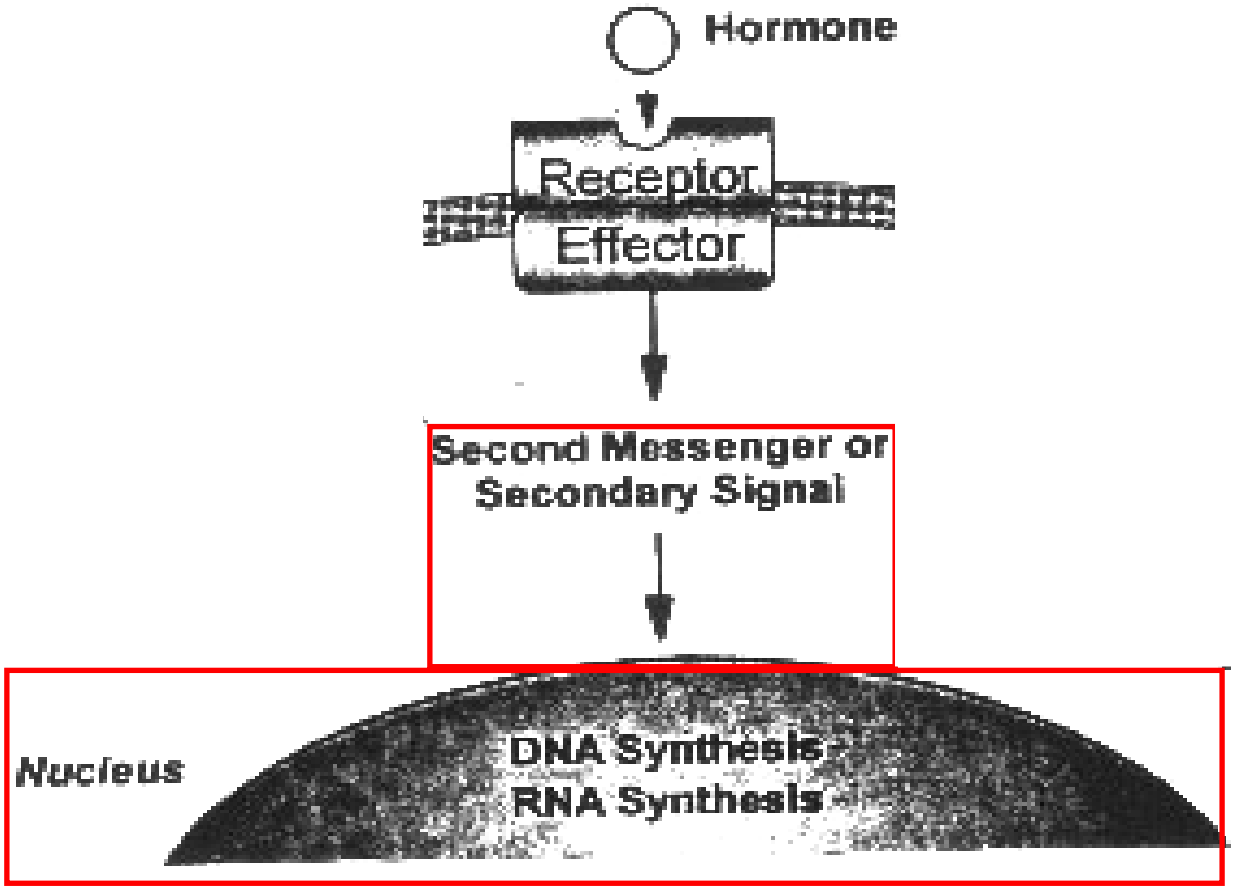
cGMP

cGMP is produced by Guanylate cyclase which catalyzes the conversion of GMP to cyclic GMP.

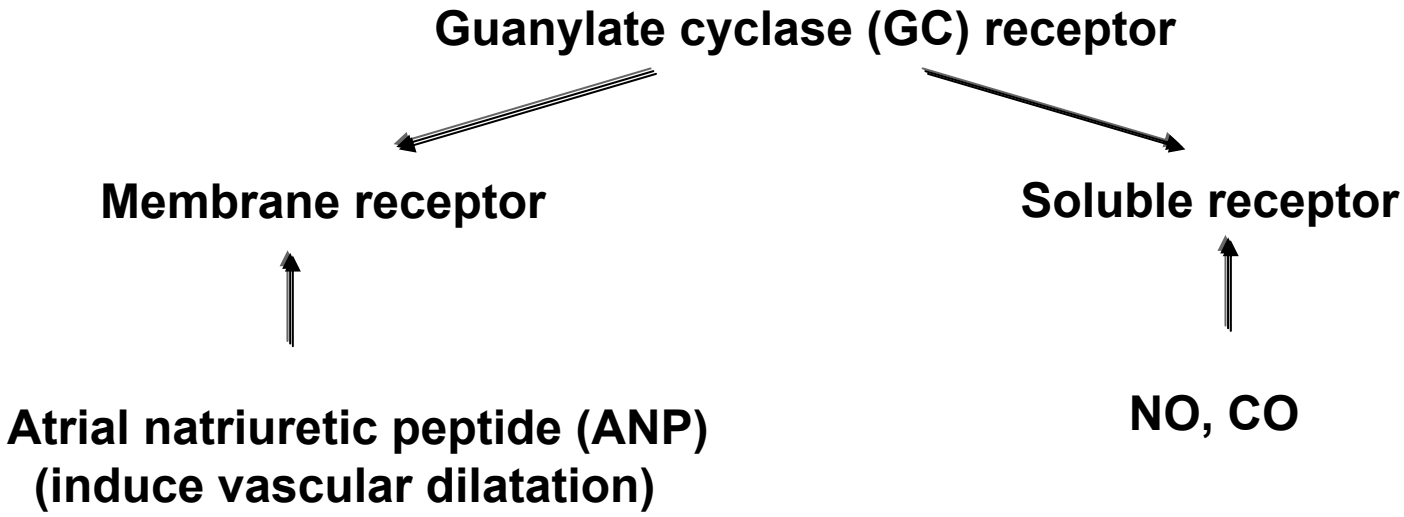
The intracellular targets of cGMP are:

cGMP-dependent protein kinases (G-kinases);
cGMP-regulated phosphodiesterases;
cGMP-gated ion channels.

Second messengers such as calcium and cGMP regulate multiple cellular processes



Regulation of gene expression by cyclic GMP



Since the focus of this lecture series is primarily on regulation of gene expression, we shall skip the activities of soluble GC receptors

How does ANP regulate gene expression by cGMP pathway?

ANP binds to its receptor and stimulates its intracellular guanylyl cyclase (GC) domain.

The ANP receptor has been named natriuretic peptide receptor 1 or guanylyl cyclase A (NPR1/GCA), a 130-kDa transmembrane protein that converts GTP cGMP.

The active NPR1/GCA receptor is a homodimer containing an extracellular ANP-binding domain at its aminoterminal end and an intracellular GC domain at its carboxy-terminal end.

cGMP molecules thus synthesized, bind to target proteins, including the cGMP-dependent protein kinases (PKG) I and II, the cyclicnucleotide gated ion channels and the cyclic nucleotide phosphodiesterases (PDEs).

On activation by cGMP, PKG phosphorylates proteins such as CREB, ATF-1 which in turn activate the expression of target genes.

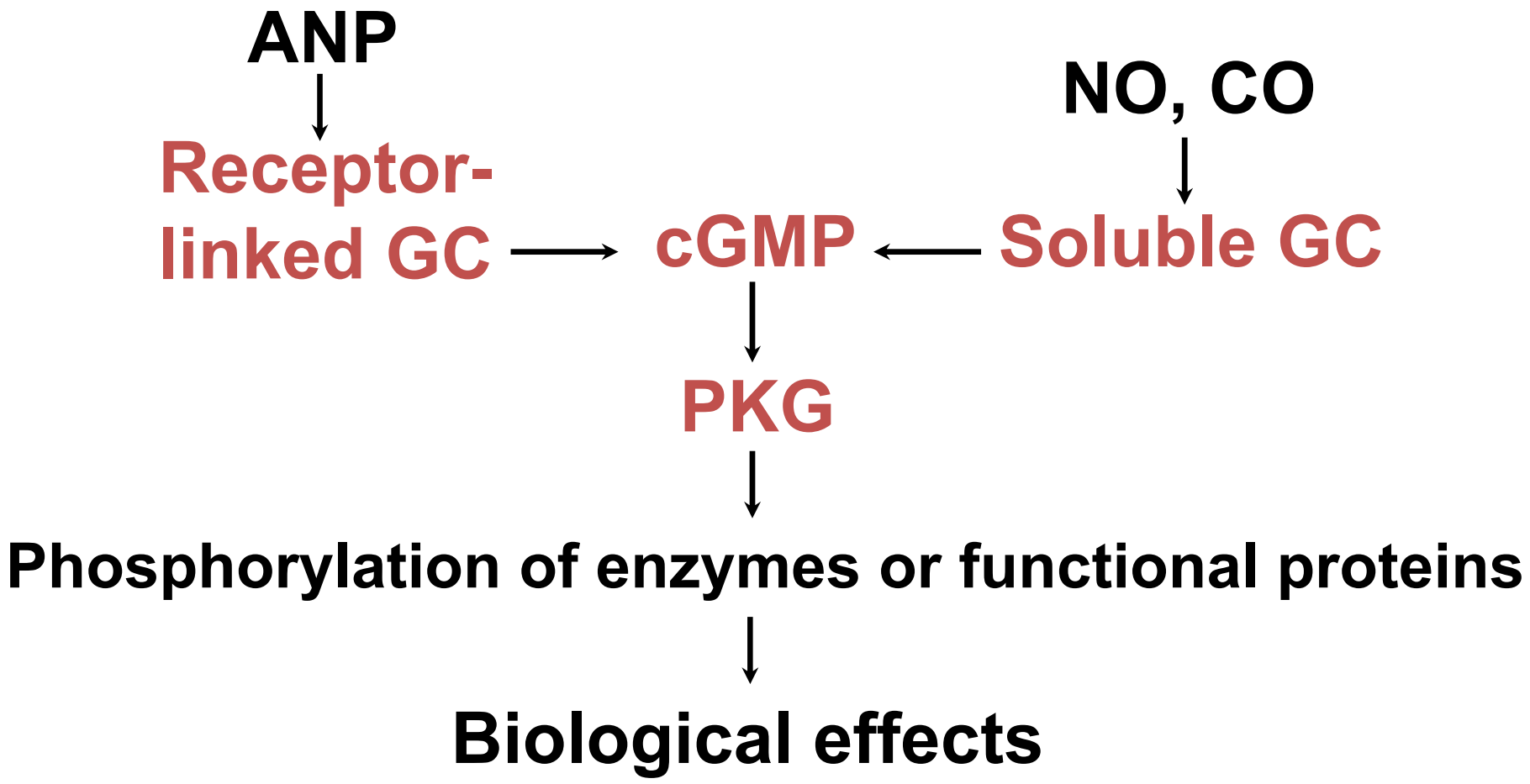
http://www.jbc.org/cgi/doi/10.1074/jbc.M109.061622

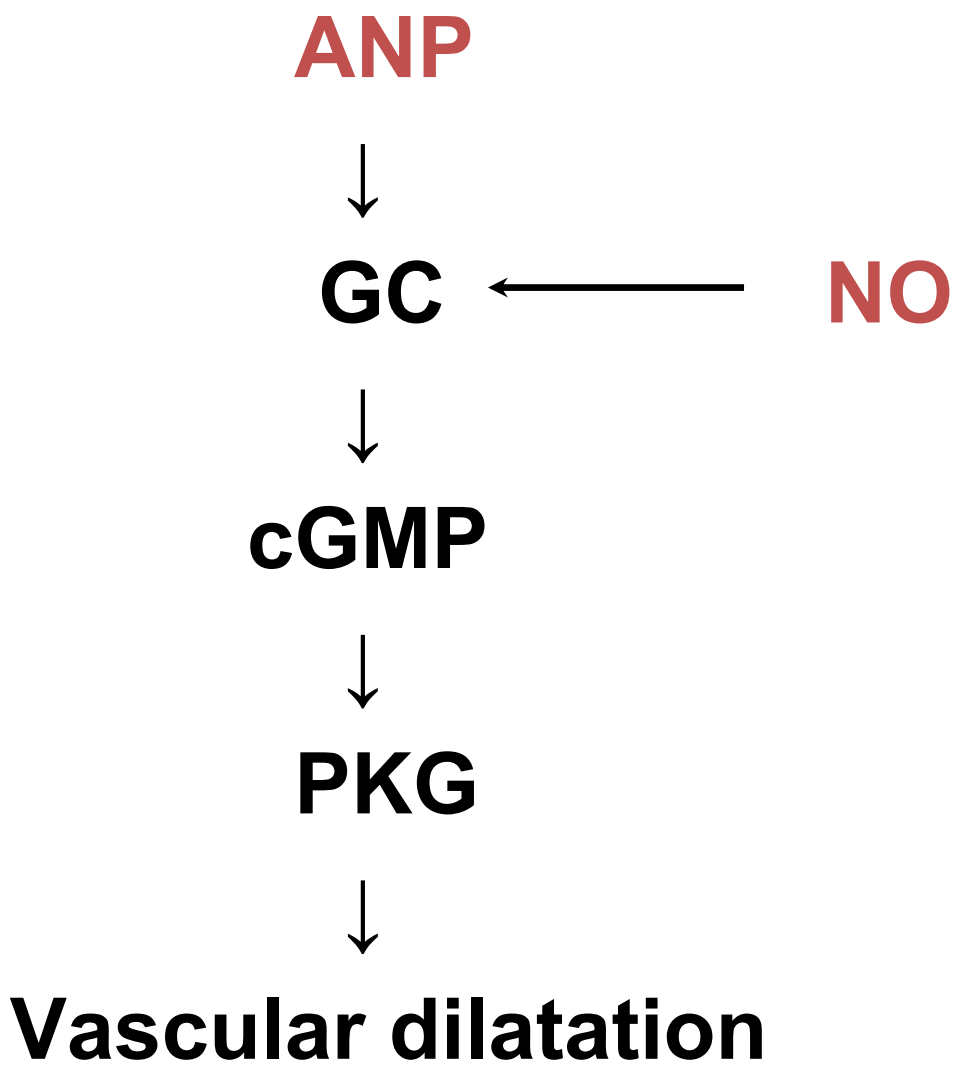
Transcriptional regulation by cGMP can also occur indirectly.

Indirect control affects upstream signalling pathways modulating specific targets.

For example, cGMP activates mitogen-activated protein (MAP) kinases which regulate several transcription factors, such as CREB, ternary complex factor, activating transcription factor-2 (ATF-2) and c-jun.

cGMP-dependent PKG pathway




ANP
GC
NO
cGMP
PKG
Vascular dilatation

Intracellular cyclic GMP concentrations are regulated by the action of guanylyl cyclases and by the rate of degradation by cyclic GMP-specific phosphodiesterases,

NO activates soluble guanylyl cyclase, which in turn catalyzes the formation of cyclic GMP from GTP, whereas cyclic GMP-specific phosphodiesterases catalyze the hydrolysis of cyclic GMP to GMP, ending signal transduction.

Phosphodiesterase 5, phosphodiesterase 6 and phosphodiesterase 9 are specific for cyclic GMP

Sildenafil (Viagra®), a novel inhibitor of the cyclic GMP-specific phosphodiesterase 5, increases intracellular concentrations of cyclic GMP and thus is effective in the clinical management of erectile dysfunction.

Terrett et al., Sildenafil (Viagra™), a potent and selective inhibitor of type 5 cGMP phosphodiesterase with utility for the treatment of male erectile dysfunction. *Bioorg. Med. Chem. Lett.* **6** (1996), pp. 1819–1824.

Boolell et al., Sildenafil, a novel effective treatment for male erectile dysfunction. *Br. J. Urol.* **78** (1996), pp. 257–261.

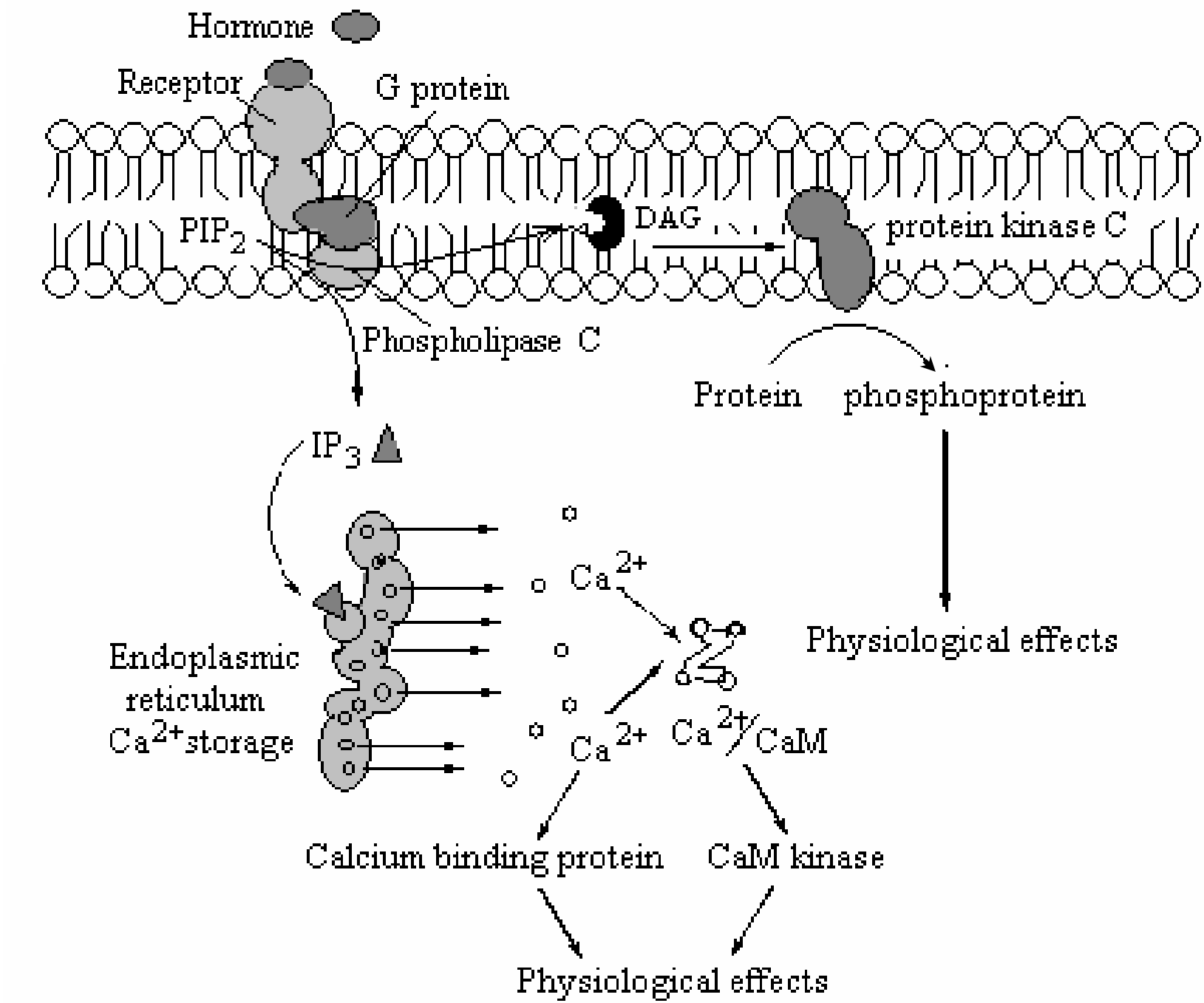
In the central nervous system, the nitric oxide (NO)-sensitive sGC isoform is the major enzyme responsible for cGMP synthesis.

Phosphodiesterases (PDEs) are enzymes for hydrolysis of cGMP in the brain, and they are mainly isoforms.

The NO/cGMP signaling pathway has been shown to play an important role in the process underlying learning and memory.

Aging is associated with an increase in PDE expression and activity and a decrease in cGMP concentration.

LIPID MOLECULES
AS
SECOND MESSENGERS


Hormone
Receptor
G protein
PIP2
DAG
protein kinase C
Phospholipase C
Protein
phosphoprotein
IP3
Ca2+
Endoplasmic
reticulum
Ca2+storage
Physiological effects
Ca2+
Ca2+/CaM
Calcium binding protein
CaM kinase
Physiological effects

Hydrolysis of PIP2 generates
Diacylglycerol (DAG) and Inositol 3 Phosphate (IP3)

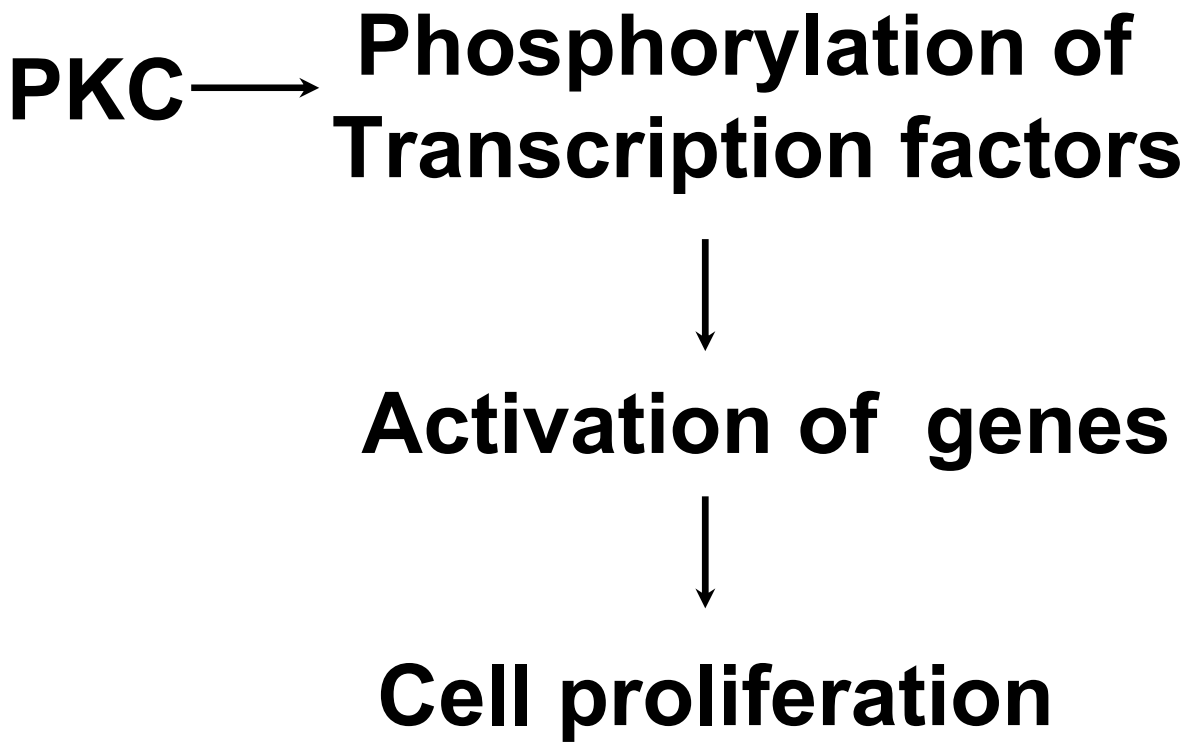
DAG together with Ca++ activates Protein Kinase C
IP3 is involvedin Calcium mobilization

Functions of PKC

- **regulation of metabolism**

PKC →Ser/Thr-P

- **Gene expression**


PKC
Phosphorylation of
Transcription factors
Activation of genes
Cell proliferation

PIP2 derived second messengers

Phospholipase A2 (PLA2) – arachidonic acid
PLC – DAG/IP3 or ceramide
PLD – phosphatidic acid

Cellular effects

- Mostly through activation of PKC
- Gene expression
- Cell motility
- Production of next generation of second messengers

Hormones that act through phospholipid breakdown

- Epinephrine and norepinephrine (α-adrenergic receptors)
- Acetylcholine (muscarinic receptor)
- TNFα

PIP2 breakdown

- Second messenger system for Gq family of G proteins
- Gq activates phospholipase C (PLC)
- Phospholipase C (PLC) is membrane bound enzyme
- PLC β breaks phoshatidylinositol 4,5 bisphosphate (PIP2) to IP3 and DAG

PIP2 breakdown

- Both IP3 and DAG are second messengers
- IP3 increases intracellular calcium levels via the release from intracellular stores such as ER
- DAG activates protein kinase C (PKC)

Protein kinase C (PKC) signaling

- Serine/threonine kinase
- Activated by DAG
- Phosphorylates various cellular effectors
- Activates transcription factors AP-1 (c-fos and cjun - both protooncogenes)

Lipid derived second messengers for intercellular signaling

- Eicosanoids
- Arachidonic acid derivatives
- Prostaglandins
- Thromboxanes
- Leukotrienes
- Lipoxins

Lipid derived second messengers for intercellular signaling

- Escape the cell and participate in intercellular signaling
- Sensitize normal physiological responses
- Important as local inflammatory mediators

Membrane Receptor signalling

1. Receptors that regulate cyclic AMP formation
2. Receptors that regulate cyclic GMP formation
3. Receptors that regulate diacylglycerol (DAG) and inositol triphosphate (IP3)
4. **Receptors that possess protein kinase activity**

Regulation of gene expression
by
Protein Kinase C

NEXT CLASS